

AD _____

Award Number: DAMD17-99-1-9193

TITLE: Characterization of Early Genomic Changes in Mammary
Glands of High Risk Women

PRINCIPAL INVESTIGATOR: Robert Dickson, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20057

REPORT DATE: July 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

DTIC QUALITY INSPECTED 4

20010122 144

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 074-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE July 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 99 - 30 Jun 00)		
4. TITLE AND SUBTITLE Characterization of Early Genomic Changes in Mammary Glands of High Risk Women			5. FUNDING NUMBERS DAMD17-99-1-9193	
6. AUTHOR(S) Robert Dickson, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University Medical Center Washington, DC 20057 E-MAIL: dicksonr@gunet.georgetown.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES Report contains color photos				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Because many of the familial breast cancer patients carry a mutation in <i>brca-1</i> on chromosome 17 or <i>brca-2</i> on chromosome 13, the first genetic event that may occur in their mammary glands to begin the progression toward cancer is on one of these two chromosomes. This genetic event is termed loss of heterozygosity (LOH). It is unknown if these genetic changes correspond to a recognizable histopathological abnormality, nor what are the precise associated chromosomal changes leading to cancer. At the Lombardi Cancer Center, we have a large ongoing study to test high-risk women for <i>brca</i> mutations and to counsel them on their prevention options. One of the options is prophylactic mastectomy, and our Histopathology and Tissue Shared Resource is maintaining a repository and database, with associated blood specimens of such material. We now propose to utilize these "normal glands" removed for preventive purposes, as well as <i>brca</i> -positive breast cancer and associated mastectomy tissue to learn more about the precise earliest histopathologic changes and associated chromosomal changes in <i>brca</i> -carriers. From these studies we hope to learn more about the natural history of <i>brca</i> positive breast lesions and to improve their molecular diagnosis and the decision making for the patients.				
14. SUBJECT TERMS Breast Cancer			15. NUMBER OF PAGES 12	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

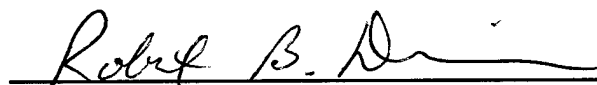
N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.


PI - Signature

Date

4/25/00

Table of Contents

Cover.....	1
SF 298.....	2
Foreword.....	3
Table of Contents.....	4
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	7
References.....	8
Appendices.....	9-12

Introduction:

A major advance in breast cancer research over the past few years has been the identification of genes which are responsible for hereditary breast cancer, namely *BRCA1* and *BRCA 2* genes (1, 2). However the actual roles of these two genes in breast tumorigenesis are not clear. For example, the basis of the vast variation in penetrance of different mutations in these two genes, variations of the same mutation in different individuals of a similar family and variations among families, are not clear. We would like to begin to understand the earliest steps in histopathologic appearance and potentially associated genomic alterations as breast cancer begin to arise in high risk, *BRCA* -carrying individuals. No studies to date have systematically examined the early consequences of inheritance of a mutation in the *BRCA-1* or *BRCA-2* genes for corresponding early changes in breast histopathology. In addition, no studies have addressed the correlation of such early abnormalities in the breasts of *BRCA* mutation carriers with genomic gains, losses, loss of heterozygosity (LOH), or replication error repair instability. Studies of morphologically normal lobules adjacent to sporadic breast cancer have shown the presence of LOH in these morphologically "normal" tissues suggesting the presence of a "field effect" of preexisting genomic damage in the gland, which gives rise to the tumor (3). Two recent studies have shown cytogenetic abnormalities in prophylactic mastectomy specimens characterized by hyperplasia without atypia, from patients with a positive family history of breast cancer (unknown *BRCA* status) (4, 5). Taken together, these studies suggest that there may be detectable early genomic changes in the breasts of high risk patients with inherited predisposition to breast cancer. Characterization of such changes may provide further insight into the onset and progression of breast tumors in these patients.

In this project we are evaluating the early genomic changes that occur in the mammary glands of patients with increased predisposition to breast cancer because of germline mutations in the *BRCA-1* or *BRCA-2* genes. To address this question we are analyzing mammary tissues from a group of patients with such mutations. Our analysis will include tumor tissues from patients with breast cancer, normal surrounding tissues to breast tumors, contralateral prophylactic mastectomy samples from patients with breast tumors and prophylactic mastectomy samples from patients with no breast tumors. Our evaluation of these samples is done using a combination of molecular tests and expert pathology review. Following careful evaluation of each sample by pathologist, we will study loss of heterozygosity (LOH) at loci on chromosome 17 in patients with *BRCA-1* mutations and on chromosome 13 in patients with *BRCA-2* mutations. In addition, a genome wide search for chromosomal gains and losses will be conducted on all samples using comparative genomic hybridization (CGH). The overall purpose of this work is to learn more about the natural history of *BRCA* - positive breast lesions and to improve the molecular diagnosis of *BRCA* malignancy- associated changes. These studies should aid to improve early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk.

Body:

During the first year of this project we have actively proceeded to achieve the groundwork for the success of this study. Specifically, we have recruited Dr. Luciane Cavalli, a postdoctoral fellow with an excellent experience in cytogenetics and molecular biology. Dr. Cavalli started her fellowship in October 1999. Her CV is attached. Progress relative to our statement of work (revised and previously approved according to reviewers' suggestions) is as follows.

Patient accrual:

Over the past year, we have continued to collect tissues from high risk patients with known *BRCA* status. At present we have accrued 15 patients with *BRCA* mutations: 10 patients with *BRCA-1* mutations and 5 patients with *BRCA-2* mutations. (This number represents the subset of the high risk patients who have *BRCA-1/2* mutations).

Pathology review of the *BRCA* 1/2 positive patients:

Tissue is available from 14 of 15 cases:

Sclerosing adenosis and cystic change are present in all fourteen cases. Usual ductal hyperplasia is seen in seven and atypical ductal hyperplasia in one case. Atypical lobular proliferation is seen in one case. Fibroadenomatoid hyperplasia is present in three cases. A small duct papilloma is seen in one case.

Laser capture microdissection (LCM):

Dr. Cavalli, with the assistance of our pathologist, Dr. Baljit Singh, was trained to use the LCM system which is available in our tumor bank. She has spent several hours of training using sporadic breast tumor samples available from our tumor bank. In addition Dr Cavalli has established a contact with the NIH LCM core facility, directed by Drs. Robert Bonner and Lance Liota. She has visited the facility and had the chance to interact with the core staff and discuss the protocol they follow. This interaction is very helpful to our group because it gives us access to the lab that discovered and developed the LCM technique. In June 2000, Drs. Haddad, Singh and Cavalli attended the LCM symposium held at NIH where several presentations and discussions took place from different groups using this approach. The meeting allowed very helpful interactions to take place among users and experts in the field. At present, Dr. Cavalli is well trained to use LCM on our *BRCA-1/2* samples and will be starting this in the second year of the grant.

LOH studies:

The PCR conditions to study several of the LOH loci on chromosomes 13 and 17 which are needed for this project have been established and validated using DNA extracted from blood and from tumor tissues. We are presently in the process of optimizing the conditions for DNA obtained from laser capture microdissected (LCM) specimens.

To achieve that optimization, we are using sporadic breast samples which are available from our tumor bank. Once this is completed, we will test our 15 *BRCA-1/2* mutation positive samples (plus all newly accrued patients).

Figure 1 shows LOH analysis performed in our lab on samples from one of our patients with a *BRCA-1* mutation and breast cancer for the locus D17S855 (a *BRCA-1* intragenic locus).

Analysis of DNA prepared from the patient's lymphocytes (Fig 1, "Blood") and the normal breast tissues (prophylactic mastectomy) (Fig 1, "Normal Breast") show 2 peaks representing 2 alleles while the DNA from the breast tumor (Fig 1, "Breast tumor") show loss of heterozygosity for that locus.

CGH evaluation:

CGH analysis using fresh tissues and/or archival formalin-fixed, paraffin-embedded tumors is routinely performed in our lab. Dr. Cavalli was trained to use this technique and will apply it to evaluate our samples. Figure 2 shows the analysis from the breast tumor described in the previous paragraph from the same patient with *BRCA-1* mutation. The profile shows both gains and losses of chromosomal material. The five vertical lines on the right side of the chromosome ideograms reflect different values of the fluorescence ratio between the test DNA prepared from tumor cells and the control DNA. The values are 0.5, 0.75, 1, 1.25 and 1.5 from left to right. The ratio profile (curve) was computed as a mean value of at least 8 metaphase spreads (n is the number of chromosomes used to generate each ratio profile). Gains are noticed on chromosomes 6q, 9, 10p, and 11q, amplification on chromosome 8q and losses on chromosomes 4, 8p and 18. We plan to perform this assay on all our samples during the second year of the project.

Revised statement of work:

Year 1: In the first year, we will obtain hereditary breast tumors with associated mastectomy tissue as well as prophylactic mastectomies from *BRCA* carriers. [Completed]. We will also fully establish and validate all necessary LOH assays, following pathologic review of all specimens, for comparison of their genomic changes relative to nearby pathologically reviewed and microdissected non-tumor tissue (Aims 1 and 2). [Completed].

Year 2: In the second year, specimen collection will continue, Aims 1 and 2 will continue, and Aims 3 and 4 (study of pathologically reviewed contralateral prophylactic mastectomy tissues and pathologically reviewed bilateral prophylactic mastectomy tissues) will begin.

Year 3: In the third year, all 4 aims will be completed and data analyzed. Specifically, pathologic diagnosis will be correlated with genomic and chromosomal changes for each aim.

Key Research Accomplishments:

- Accrued 15 patients with *BRCA 1/2* mutations who underwent mastectomy and/or prophylactic mastectomy.
- Reviewed tissues by an expert pathologist.
- Optimized conditions for LOH studies using DNA from both lymphocytes of patients with *BRCA 1/2* mutations and LCM samples from archival tumors.
- Optimized conditions for CGH studies of archival breast tumor tissues from *BRCA 1/2* mutation carriers.

Reportable outcomes: N/A

Conclusions:

In this first year we have established the groundwork for the success of this project. As described in our "revised statement of work", we have obtained hereditary breast tumors with associated mastectomy and prophylactic mastectomy tissues from *BRCA* carriers. We established and validated the necessary LOH assays. A pathologist evaluated the tissues and helped in establishing the LCM technique in our lab. We will now address our primary hypothesis: Genomic changes (genomic gains, losses, LOH, or replication error repair instability) may be

detected in the histologically abnormal, premalignant and malignant regions in the breasts of *BRCA* carriers. These changes may also be present in tissues adjacent to *BRCA*- associated cancer and in prophylactic mastectomy specimens, thus representing the *earliest* detectable changes. In the upcoming two years we will look for these changes in our collection of specimens. *These data should aid in improved early detection and diagnosis of hereditary breast cancer and provide more information when considering prevention strategies for such women at risk.*

References:

- 1- Miki, Y., Swensen, J., Shattuck-Eidens, D., *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA-1*. Science, 266:66-69, 1994.
- 2- Wooster, R., Bignell, G., Lancaster, J., *et al.* Identification of the breast cancer susceptibility gene *BRCA-2*. Nature, 378:789-796, 1995
- 3- Deng, G., Lu, Y., Zlotnikov, G., Thor, AD. and Smith, HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Science, 274:2057-2059, 1996.
- 4- Teixeira, MR., Pandis, N., Gerdes, AM., *et al.* Cytogenetic abnormalities in an in situ ductal carcinoma and five prophylactically removed breasts from members of a family with hereditary breast cancer. Breast Cancer Res Treat, 38:177-182, 1996.
- 5- Petersson, C., Pandis, N., Mertens, F., *et al.* Chromosome aberrations in prophylactic mastectomies from women belonging to breast cancer families. Genes Chromosomes Cancer, 16:185-188, 1996.

Appendices:

CV of Dr. Cavalli
Figure 1. LOH
Figure2. CGH

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE		
Luciane Regina Cavalli	Postdoctoral Research Fellow		
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (If applicable)	YEAR(s)	FIELD OF STUDY
Federal University of Parana, Brazil	BS	1986-89	Biological Sciences
Federal University of Parana, Brazil	Masters	1990-94	Cancer Cytogenetics
Federal University of Parana, Brazil	PhD *	1995-99	Molecular Biology
* experimental part : University of Colorado, Denver, CO, USA			
Georgetown University Medical Center (Institute for Molecular and Human Genetics)	post doctoral	oct 1st / 99-present	Molecular Cytogenetics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

PROFESSIONAL EXPERIENCE

1990-91: English teacher at the Open English House - Curitiba, Parana, Brazil
1993-95 / 1997-99 : Biologist at the Laboratory of Human Cytogenetics at the Clinical Hospital of the Federal University of Parana, Brazil

PUBLICATIONS

1. Cavalli LR, Rogatto SR, Rainho CA, Santos MJ, Cavalli IJ, Grimaldi DM. Cytogenetic report of a male breast cancer. Cancer Genetics and Cytogenetics 81: 66-71 (1995).
2. Cavalli LR, Cavalieri LMB, Ribeiro LA, Cavalli IJ, Silveira R, Rogatto SR. Cytogenetic evaluation of 20 primary breast carcinomas. Hereditas 126: 261-268 (1997).
3. Cavalli LR, Varella-Garcia M, Liang BC. Diminished tumorigenic phenotype after depletion of mitochondrial DNA. Cell Growth&Differentiation 8: 1189-1198 (1997).
4. Cavalli LR, Liang BC. Mutagenesis, tumorigenicity and apoptosis: Are the mitochondria involved ? Mutation Research 398: 19-26 (1998).
5. Cornelio DA, Schmid-Braz AT, Cavalli LR, Lima RS, Cavalli IJ. Cytogenetic alterations observed in a gynecomastia case. Cancer Genetics and Cytogenetics 115: 128-133 (1999).

LECTURES AND COURSES (last three years)

1. Genetic and Cytogenetic of Breast Tumors. At the Oncology Surgery Service of the Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil, December, 1997.
2. Cytogenetic and Molecular Genetics of Hematologic Diseases and Solid Tumors. At the IV Genetic Meeting of the state of Paraná, Londrina, Paraná, Brazil, June, 1998.

- 3. Molecular and Cytogenetic Aspects of the Human Neoplasias. IV Genetic Meeting of the state of Paraná, Londrina, PR, Brazil, June, 1998.
- 4. Genetic and Cytogenetic of Breast Tumors. At the Oncology Surgery Service of the Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil, September 1st, 1998.
- 5. Cytogenetics and Molecular Genetics of Cancer. At the Biology Course of the Catholic University of Paraná, Curitiba, PR, Brazil. August 31st – September 4th, 1998.
- 6. Introduction to Molecular Cytogenetic Techniques (FISH, CGH, SKY). At the Biology Course of the Catholic University of Paraná, Curitiba, Paraná, Brazil. August 31st – September 4th, 1998.

RELEVANT ABSTRACTS (last 3 years)

1. **Cavalli LR**, Liang BC. Reduction of the tumorigenic phenotype in rho0 human breast cancer cells. 88th Annual Meeting of the American Association for Cancer Research (AACR), San Diego, California, EUA, 1997. (Proceedings of the AACR, vol38, march, 1997)
2. **Cavalli LR**, Varella-Garcia M, Liang BC. Elimination of the mitochondrial DNA - reduction of the tumorigenic phenotype in mammary carcinoma. 43rd National Congress of Genetics, Brazil, 1997 (Rev.Brasileira de Genética, 20 (3), 1997)
3. Schmid-Braz AT, Cornélio DA, **Cavalli LR**, Silveira R, Cavalli IJ. Cytogenetic evaluation of an ovarian mature teratoma. 43rd National Congress of Genetics, Brazil, 1997 (Rev.Brasileira de Genética, 20 (3), 1997)
4. Cornélio DA, Schmid-Braz AT, **Cavalli LR**, Freitas A, Silveira R, Cavalli IJ Cytogenetic study in mammary carcinomas. 43rd National Congress of Genetics, Brazil, 1997 (Rev.Brasileira de Genética, 20 (3), 1997)
5. Schmid-Braz A, Cornélio DA, **Cavalli LR**, Lima RS, Cavalli IJ. Cytogenetic study in ovarian tumors. 44th N National Congress of Genetics, Brazil, September, 1998 (Genetics and Molecular Biology, 21 (3), 1998)
6. Cornélio DA, Schmid-Braz AT, **Cavalli LR**, Lima RS, Cavalli IJ. Cytogenetics alterations in a gynecomastia case. 44th National Congress of Genetics, Brazil, September, 1998 (Genetics and Molecular Biology, 21 (3), 1998)
7. **Cavalli LR**, Cornélio DA, Schmid-Braz AT, Wuicik L, Lima R, Rogatto SR, Cavalli IJ. Correlation between karyotypic findings and clinicopathologic features in primary breast tumors". 48th Annual meeting of the American Society of Human Genetics, Denver, CO, USA, October, 1998 (The American Journal of Human Genetics , 63 (4), 1998)
8. Haddad BR, Rone J, **Cavalli LR**, Toyota M, Issa JPJ. Correlation of Chromosomal Instability with CpG island methylation status in colorectal tumors. AACR 91st. Annual Meeting, April 1-5, 2000, San Francisco, CA, USA.

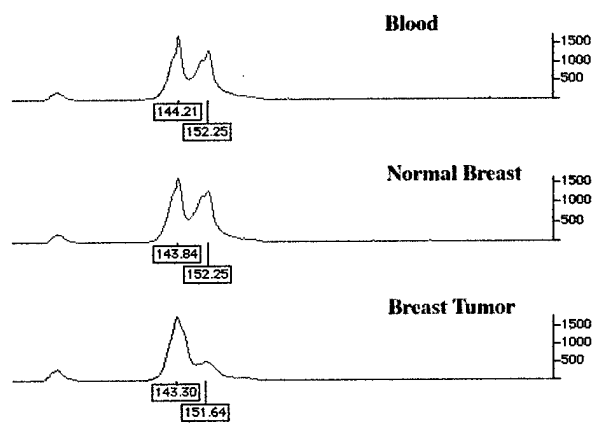


Figure 1

